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Strain-Specific Differences in LFA-1 Induction on Measles Virus-Infected Monocytes and Adhesion and Viral Transmission to Endothelial Cells

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Measles virus (MV) infection of monocytes induces leukocyte function-associated antigen-1 (LFA-1), an integrin that mediates intercellular adhesion to the endothelium. Thus, an increase in LFA-1 expression could lead to enhanced monocyte adherence and virus dissemination to endothelial cells (ECs) and potentially be an important means of distinction between MV strains. We identified both vaccine and wild-type strains that induced LFA-1 and others that failed to induce. Although adhesion of MV-infected monocytes and viral transmission to ECs was demonstrated, strain-specific differences were not correlated with LFA-1 induction. MV infection of ECs was dramatically reduced in the absence of cell contact, suggesting virus dissemination by cell-cell transmission.

Measles virus (MV) is a negative-strand, enveloped RNA virus that is a member of the *Morbillivirus* genus in the *Paramyxoviridae* family. The primary cell infected in blood is the monocyte (8), and infected monocytes may be responsible for dissemination of virus from the respiratory tract, as occurs in animal models of MV infection (15). During the secondary viremia phase of measles, endothelial cells (ECs) of small vessels throughout the body are infected, and this is often accompanied by mononuclear infiltration and infection of surrounding tissue (for a review, see reference 10). At present, it is unknown whether dissemination to ECs results from infection with cell-free virus or from interactions with circulating MV-infected cells.

MV infection of monocytes, in vitro, induces the surface expression of leukocyte function-associated antigen-1 (LFA-1) (1, 26), a member of the integrin family of cell adhesion molecules. LFA-1, by binding to its major counterreceptor, the intercellular adhesion molecule-1 (ICAM-1), on ECs, mediates cell-cell adhesion that promotes leukocyte recruitment to the endothelium and the subsequent migration through EC junctions. Thus, an increased expression of LFA-1 on monocytes could lead to enhanced adherence to ECs and contribute to virus dissemination.

The suggestion has been made that wild-type (wt) strains of MV are better modulators of LFA-1 expression than attenuated vaccine strains (1, 26), which is potentially an important distinction between these strain types for enhanced cell-cell interactions and dissemination of virus. However, previous LFA-1 studies have analyzed only a limited number of strains (1, 2, 22, 26). In this study, we examine the induction of LFA-1 by a broad panel of MV strains. We identified both wt and vaccine strains that induced LFA-1 and others that failed to induce. We also evaluated the adhesion of MV-infected monocytes and viral transmission to ECs to determine whether a relationship exists between the abilities of different MV strains to alter LFA-1 expression and their pathogenic potential.

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LFA-1 expression in MV-infected monocytes. U937 cells were maintained in suspension culture in RPMI 1640 supplemented with 10% fetal calf serum (FCS; HyClone, Logan, Utah) and PSG (100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mM L-glutamine [GIBCO BRL, Grand Island, N.Y.]). The Moraten (MOR) (12), Zagreb (ZAG) (13), and CAM-70 (CAM) (24) live attenuated strains were supplied as lyophilized vaccines and propagated in African green monkey kidney Vero cells as previously described (19). The jm77 (18), Chi1 (18), and Pa2 (20) wt strains were isolated from unvaccinated individuals with measles and were minimally passaged five to eight times in Vero cells. The Ph26 wt strain (9) was passaged extensively, however, before propagation in Vero cells in our laboratory. U937 cells were infected at a multiplicity of infection of 0.1 as detailed elsewhere (2). At 24-h intervals postinfection (p.i.), 5×10^5 cells were washed once each in phosphate-buffered saline (PBS; GIBCO BRL) with 5% FCS (PBS–5% FCS) and in versene (GIBCO BRL), before incubation on ice with fluorescein isothiocyanate-conjugated murine monoclonal antibodies (MAbs; Ancell, Bayport, Mich.). The MAb 81-I-366, prepared against the MV hemagglutinin (H) protein (3), was not directly conjugated, and therefore, a second incubation step was performed with a fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G (IgG) antibody (Southern Biotechnology Associates, Inc., Birmingham, Ala.). Stained cells were washed three times with PBS–5% FCS and resuspended in a 1% paraformaldehyde solution (Sigma, St. Louis, Mo.) before analysis, with appropriate gating, on a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.).

The U937 human monocytic cell line was used to assess LFA-1 expression, since changes in U937 cell surface proteins were reported to be consistent with those in freshly isolated peripheral blood mononuclear cells (1). Surface expression of LFA-1 was monitored from 24 to 96 h p.i. by fluorescence-activated cell sorting (FACS) analyses (Table 1). Greater than 90% of mock- and MV-infected U937 cells were positive for LFA-1 expression throughout the time course of infection. An increase in the mean channel values of fluorescence intensity (MFI) of cells stained for LFA-1 was observed only upon

TABLE 1. Surface expression of LFA-1 on mock- and MV-infected U937 cells[a]

Infecting strain and MAb	Expression determined at:							
	24 h		48 h		72 h		96 h	
	MFI	% (+)	MFI	% (+)	MFI	% (+)	MFI	% (+)
None (mock infection)								
IgG	3.24	1.06	3.35	1.41	2.79	1.37	2.94	2.15
LFA-1	30.89	98.26	33.49	98.63	32.92	98.37	26.29	92.63
MOR								
IgG	4.32	1.44	4.09	1.93	4.36	3.27	4.36	5.18
LFA-1	31.21	98.58	40.19	99.24	45.2	99.16	49.1	98.88
ZAG								
IgG	3.6	2.16	5.33	9.06	4.34	6.43	4.74	7.54
LFA-1	31.69	97.89	47.16	98.12	43.99	97.44	44.72	93.49
CAM								
IgG	ND[b]	ND	ND	ND	2.08	0.52	4.85	8.01
LFA-1	ND	ND	ND	ND	29.07	95.63	27.74	95.34
Ph26								
IgG	4.86	2.7	5.57	6.28	4.76	6.09	7.99	20.12
LFA-1	35.10	98.11	45.77	98.89	53.33	98.91	73.75	99.01
jm77								
IgG	ND	ND	ND	ND	2.13	0.69	5.27	8.24
LFA-1	ND	ND	ND	ND	29.86	95.86	27.24	93.92
Chi1								
IgG	3.79	0.79	4.41	0.6	2.96	0.71	2.92	1.39
LFA-1	30.26	97.6	31.57	98.71	31.63	98.27	26.17	92.75
Pa2								
IgG	4.02	1.88	3.83	1.99	3.03	1.41	3.07	1.84
LFA-1	30.06	98.69	32.05	98.51	31.83	97.77	26.16	91.85

[a] U937 cells were infected with various MV strains, and the surface expression of LFA-1 at four time points p.i. was determined by FACS analysis. A mouse IgG isotype MAb was used as a negative control in parallel with a MAb specific for the alpha chain (CD11a) of the LFA-1 heterodimer. U937 cells were also mock infected with a Vero cell lysate. The MFI and percentages of positive-fluorescing cells [% (+)] shown are typical of an individual experiment.

[b] ND, not done.

infection with the MOR and ZAG vaccine strains and the Ph26 wt. This was illustrated by the shift to the right in the FACS-generated histograms shown in Fig. 1. In contrast, there was no LFA-1 induction in response to infection with the jm77, Chi1, or Pa2 wt strain and the CAM vaccine strain. A comparison of the average fold increases in MFI revealed that by 96 h p.i., the induction of LFA-1 expression in cells infected with MOR, ZAG, or Ph26 was nearly two to three times that in mock-infected cells (Fig. 2a). It should be noted, however, that the increase in LFA-1 expression that we observed with MOR differed from previous reports, which found no induction (1, 26). For all strains, infection was evaluated in immunofluorescence assays by the percentage of cells expressing the MV nucleoprotein (N), the most abundant viral protein. These values were as follows: for strain MOR, 10 to 50%; ZAG, 50 to 100%; CAM, 10 to 50%; Ph26, 50 to 100%; jm77, 10 to 50%; Chi1, 10%; and Pa2, 1 to 10%.

The MV H protein, a surface glycoprotein necessary for viral attachment to the host cell receptor, has been implicated in mediating the increased expression of LFA-1, possibly in a dose-dependent manner (2). Thus, surface MV H expression on infected monocytes was determined by FACS analyses, and the average fold increases in MFI are shown in Fig. 2b. For strains that induced LFA-1, MV H protein was expressed. However, infection with CAM led to high, sustained levels of MV H expression, without LFA-1 induction. Similarly, jm77 and Pa2 expressed MV H at levels associated with LFA-1 induction in other strains, yet LFA-1 was not induced. Increases in MV H expression were also not directly proportional to increases in LFA-1. This was most dramatically evidenced at 96 h p.i. by ZAG, which, despite having a 14-fold-higher level of MV H expression than Ph26, had a lower level of LFA-1 induction.

Monocyte adhesion to EC monolayers. Human umbilical vein ECs were isolated as previously described (17) and grown in M199 medium (BioWhittaker, Walkersville, Md.) supplemented with 20% heat-inactivated FCS (HyClone), 16 U of heparin (Elkins-Sinn, Cherry Hill, N.J.) per ml, 50 μg of endothelial mitogen (Biomedical Technologies Inc., Stoughton, Mass.) per ml, and PSG. ECs were seeded at 5×10^4 cells/well in gelatin-coated, 96-well, flat-bottom plates (Costar, Cambridge, Mass.) and cultured overnight until confluent. MV-infected U937 cells were pelleted and labeled for 30 min at 37°C in 5% CO_2 with the fluorochrome calcein-AM (Molecular Probes, Eugene, Oreg.) at a final concentration of 5 μM. Labeled cells were washed twice in RPMI 1640–1% FCS, stained with trypan blue to check viability, and resuspended at 2×10^6 cells/ml in RPMI 1640–1% FCS. EC monolayers were washed twice gently with RPMI 1640–1% FCS by using a pipette prior to the addition of 100 μl of labeled U937 cells/well (2×10^5 cells). Unlabeled, infected U937 cells were added in parallel as a measure of background fluorescence (F_b). Ad-

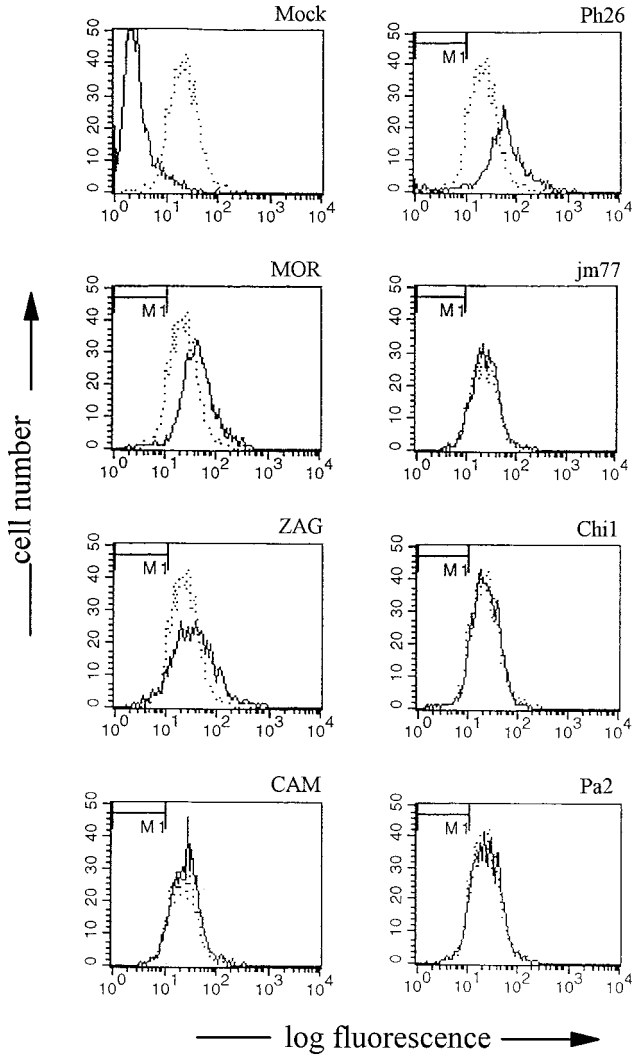

FIG. 1. Quantification of surface LFA-1 expression by FACS analysis in mock- or MV-infected U937 cells at 96 h p.i. In the panel for mock-infected cells, histograms of mouse IgG (solid line) and LFA-1 (dotted line) MAb binding are shown. In all other panels, an overlay of histograms of LFA-1 MAb binding to MV-infected (solid line) and to mock-infected (dotted line) cells is shown. M1 indicates the region excluded for data analysis based on mouse IgG MAb binding.

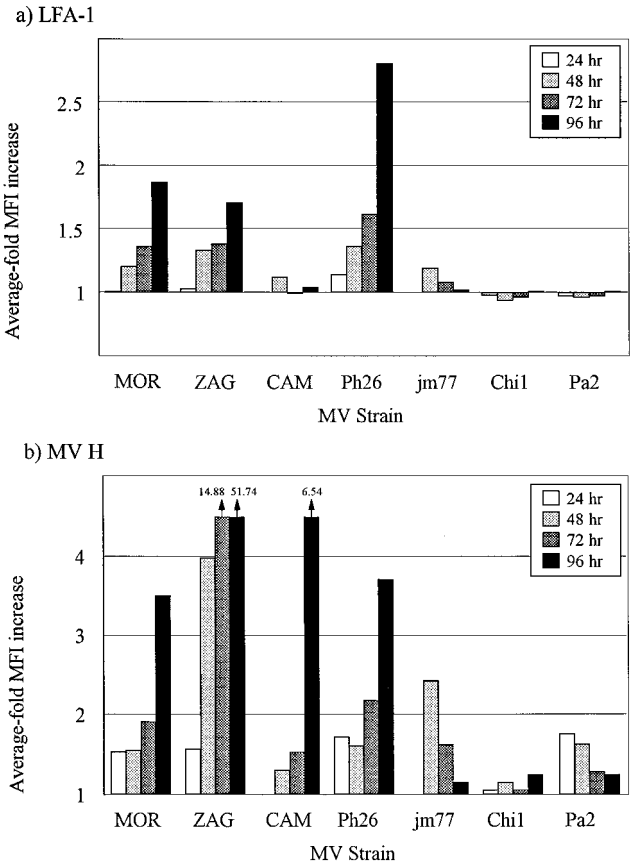

FIG. 2. Comparison of the average fold increases in MFI of MV-infected to mock-infected U937 cells by using either LFA-1 (a) or MV H (b) as the primary antibody in FACS analysis. Data shown are from duplicate experiments. Analysis of data from the 24-h time point was not done for CAM and jm77 virus strains.

hesion was allowed to occur for 30 min at 37°C in 5% CO_2. Total fluorescence (F_t) was measured at wavelengths of 485 nm (excitation) and 530 nm (emission) by using the SPECTRAFluor fluorometer (TECAN, Research Triangle Park, N.C.). Nonadherent U937 cells were removed by washing three times in 100 μl of RPMI 1640–1% FCS, and the remaining fluorescence (F_x) was measured. The percent adhesion was calculated as $[(F_x - F_b)/(F_t - F_b)] \times 100$. The data were analyzed by using a two-way analysis of variance (virus strain by run) without interaction. The mean adhesions for each strain were compared to that of mock-infected cells by using Dunnett's multiple-comparison procedure (7).

Adhesion assays were performed, using a modification of previously described procedures (4, 23, 25), to investigate whether an increase in LFA-1 expression was associated with an enhanced adhesiveness of monocytes to ECs. Although some MV-infected U937 cells were more adhesive than mock-infected cells, the enhanced adhesion did not correlate with increased LFA-1 expression (Table 2). Monocytes infected with LFA-1-inducing strains, MOR and ZAG, or noninducing strains, CAM and Chi1, were each found to be significantly more adhesive than mock-infected cells (0.05 significance level). The MV-induced adhesion was not reduced by the addition of a blocking anti-ICAM-1 MAb (data not shown).

The formation of large cellular aggregates in leukocyte cultures after MV infection (1, 22, 26) promotes virus dissemination (1), presumably by facilitating cell-to-cell contact. Both the LFA-1 (2) and MV H (2, 22) proteins have been implicated in mediating this effect. In our analyses, however, aggregation was evident only for LFA-1-inducing strains regardless of MV H expression (Table 2), even when observed for as long as 7 days p.i. (data not shown). Thus, MV-induced aggregation and adhesion may involve different mechanisms, with homotypic adhesion mediated in part by LFA-1 and heterotypic adhesion appearing to be LFA-1 independent.

Monocytic viral transmission to ECs. MV-infected U937 cells were incubated directly with ECs in RPMI 1640 growth medium or were physically separated by use of Transwell chambers fitted with a 3.0-μm-pore-size filter (Costar), which would allow for the passage of virus but not cells. Following a 24-h overlay, U937 cells (96 h p.i.) were removed and EC monolayers were washed in PBS and cultured in M199 growth medium. After an additional 24 h, ECs were harvested onto 12-well microscope slides. Immunofluorescence assays were performed on acetone-fixed cells, with a 30-min incubation at

TABLE 2. Strain-specific differences in monocyte function following MV infection[a]

Infecting strain	Aggregate size (no. of cells)[b]	LFA-1 induction (fold)[b]	MV H expression (fold)[b]	% Adhesion[c]	% ECs infected[d]	
					With contact	With no contact
None (mock infection)	≤3	1.00	1.00	33.5 ± 6.8	0	0
MOR	**5–10**	**1.87**	**3.50**	**46.9 ± 7.4**	**100**	**1**
ZAG	**5–10**	**1.71**	**51.74**	**63.3 ± 7.5**	**100**	**100**
CAM	≤3	1.04	6.54	49.4 ± 6.9	100	≤10
Ph26	**≥20**	**2.81**	**3.71**	**37.6 ± 4.1**	**100**	**<1**
jm77	≤3	1.02	1.16	37.1 ± 8.3	≤50	<1
Chi1	≤3	1.01	1.25	43.4 ± 4.9	10	<1
Pa2	≤3	1.01	1.25	34.0 ± 4.1	<1	0

[a] LFA-1-inducing strains and their results are highlighted in bold.
[b] Determinations of U937 cell aggregation, defined as greater than three attached cells, and LFA-1 and MV H surface expression, as measured by FACS analysis, after infection with various strains of MV at 96 h p.i. or mock infected with a Vero cell lysate are shown.
[c] Adhesion was evaluated at 72 h p.i. and the mean values ± standard errors of the means of duplicate experiments with three replicates per assay are shown.
[d] Coculture assays were performed at 72 to 96 h p.i., and the results shown are from replicate experiments. Values are given for ECs both with and without cell contact (see text for details).

37°C with an anti-H MAb and an anti-N MAb, 83-VII-KK2 (3), before observation by use of fluorescent microscopy.

MV could be successfully transmitted to ECs following coculture with infected U937 cells (Table 2). Since the entire EC monolayer was infected whether CAM, a noninducing strain, or any of the three LFA-1-inducing strains were used, enhanced transmission could not be associated with increased LFA-1 expression. For most strains in our panel, cell contact was critical for efficient MV transmission, since most infectious virus appeared to be cell associated and was not free to pass through the filter (Table 2). In contrast, U937 cells infected with ZAG appeared to be equally proficient at the release of infectious virus necessary to infect ECs in the absence or presence of cell contact. Transmission to ECs also occurred without direct contact with CAM-infected U937 cells, but with a 90% reduction in infectivity.

In this study, we demonstrate that LFA-1 induction cannot be used as a marker to discriminate between attenuated vaccine and nonattenuated wt strains. The level of induction that occurred in response to the MOR and ZAG vaccines and the Ph26 wt was very modest, less than a two- to threefold increase in expression, but correlated well with previous published accounts (1, 26). The unimodal shift in the FACS-generated histograms for inducing strains would suggest a generalized upregulation of LFA-1 expression in the entire cell population, not just within infected cells. In similar studies of ICAM-1 expression, its induction occurred on both MV-infected ECs and on cells that did not express viral protein (11). We also show that various MV strains interact with the monocytic cell line in a very different fashion. Most notably, the three low-passage wt strains did not appear to productively infect U937 cells, based on low or declining amounts of surface MV H expression and minimal infectious virus produced.

Our data do not demonstrate a direct association between the level of MV H protein expression and LFA-1 induction. Previous evidence that a relationship exists between MV H protein and LFA-1 modulation was suggested by nucleotide sequence comparisons of a limited set of inducing and noninducing strains (2). Moreover, the presence of phenylalanine at position 117 in the H protein (reported incorrectly as position 116 in reference 2) was hypothesized to correlate with the ability to induce LFA-1 expression (2). This amino acid cannot be used as a predictor of LFA-1 modulation, however, since strain MOR with a leucine at this position could induce LFA-1, whereas the CAM, jm77, Chi1, and Pa2 strains containing phenylalanine were ineffective. Furthermore, the Chi1 and Pa2 strains differ by only 1 and 2 residues, respectively, from the AC705 wt strain previously shown to induce LFA-1 (1, 26). Clearly, more is involved in LFA-1 induction than single amino acid substitutions within the H protein.

In our analyses, as well as in earlier studies (1, 2, 22, 26), MV strains were grown in Vero cells, the standard cell of choice for isolating and propagating measles. Recently, it has been noted that MV-induced fusion occurs more rapidly in a marmoset B-lymphocytic line (B95a) (14). Thus, the possibility exists that the cell type used for virus propagation might lead to differences in lymphotropism and affect the LFA-1 phenotype of a particular virus. However, when the CAM and Chi1 viruses were propagated in B95a cells, they failed to induce LFA-1 upon infection of U937 cells, as was observed after their passage in Vero cells (data not shown). Likewise, the procedure for virus isolation and passages in certain cell types did not lead to the selection of MV strains with differences in lymphotropism and did not influence the capacity for modulation of the human membrane cofactor protein (CD46) (21), an identified cell surface receptor for MV (6, 16). Thus, strain-specific differences in LFA-1 induction are not likely to be due to changes in the lymphotropic property of the virus during Vero cell propagation.

We investigated whether detectable differences in LFA-1 induction among MV strains were of biological significance, particularly with regard to enhanced pathogenesis. These studies indicate that strain-specific differences in the ability to induce LFA-1 did not correlate with enhanced monocyte adhesion or viral transmission to the endothelium. Indeed, many leukocyte integrins have been shown to require activation for appropriate ligand binding, with increased expression not necessarily associated with increased adhesive activity (5). Moreover, the synthesis of the MV H protein on infected ECs, and not the induction of ICAM-1, was recently shown to mediate an increased binding of monocytes (22). Viral transmission to ECs was dramatically enhanced by direct contact with MV-infected U937 cells, not LFA-1 induction. This observation that virus dissemination by cell-cell transmission is more efficient than that by cell-free virus may be especially important, in vivo, when the virus load is very low.

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REFERENCES

1. **Attibele, N., P. R. Wyde, J. Trial, S. C. Smole, C. W. Smith, and R. D. Rossen.** 1993. Measles virus-induced changes in leukocyte function antigen-1 expression and leukocyte aggregation: possible role in measles virus pathogenesis. J. Virol. **67:**1075–1079.
2. **Attibele, N. R., C. W. Smith, and P. R. Wyde.** 1995. Evidence that measles virus hemagglutinin initiates modulation of leukocyte function-associated antigen-1 expression. J. Virol. **69:**4357–4363.
3. **Bellini, W., G. Englund, K. Rammohan, and D. E. McFarlin.** 1986. Evaluation of the structural proteins of the hamster neurotropic strain of measles virus with monoclonal antibodies. J. Neuroimmunol. **11:**149–163.
4. **DeClerck, L. S., C. H. Bridts, A. M. Mertens, M. M. Moens, and W. J. Stevens.** 1994. Use of fluorescent dyes in the determination of adherence of human leucocytes to endothelial cells and the effect of fluorochromes on cellular function. J. Immunol. Methods **172:**115–124.
5. **Diamond, M. S., and T. A. Springer.** 1994. The dynamic regulation of integrin adhesiveness. Curr. Biol. **4:**506–517.
6. **Dorig, R. E., A. Marcil, A. Chopra, and C. D. Richardson.** 1993. The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell **75:**295–305.
7. **Dunnett, C. W.** 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. **50:**1096–1121.
8. **Esolen, L. M., B. J. Ward, T. R. Moench, and D. E. Griffin.** 1993. Infection of monocytes during measles. J. Infect. Dis. **168:**47–52.
9. **Frankel, J. W., H. Cooke, J. T. Deviney, M. Warner, and M. K. West.** 1957. Serological response of guinea pigs to inactivated measles virus, abstr. M2, p. 58. *In* Bacteriological proceedings 1957. American Society for Microbiology, Washington, D.C.
10. **Griffin, D. E., and W. J. Bellini.** 1996. Measles virus, p. 1267–1312. *In* B. N. Fields, D. M. Knipe, P. M. Howley, et al. (ed.), Fields virology, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
11. **Harcourt, B. H., P. A. Rota, K. B. Hummel, W. J. Bellini, and M. K. Offermann.** Induction of ICAM-1 gene expression by MV in human umbilical vein endothelial cells. J. Med. Virol., in press.
12. **Hilleman, M. R., E. B. Buynak, R. E. Weibel, J. Stokes, J. E. Whitman, and M. B. Leagus.** 1968. Development and evaluation of the Moraten measles virus vaccine. JAMA **206:**587–590.
13. **Ikic, D. M., M. Juzbasic, M. Beck, A. Hrabar, and T. Cimbur-Schrieber.** 1972. Attenuation and characterisation of Edmonston-Zagreb measles virus. Ann. Immunol. Hung. **16:**175–181.
14. **Kobune, F., H. Sakata, and A. Sugiura.** 1990. Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. J. Virol. **64:**700–705.
15. **McKendall, R. R., D. R. Carrigan, and K. P. Johnson.** 1981. Lymphoid cell infection by measles virus in newborn hamsters: role for monocytes in virus spread to distant sites. J. Neuroimmunol. **1:**261–274.
16. **Naniche, D., G. Varior-Krishnan, F. Cervoni, T. F. Wild, B. Rossi, C. Rabourdin-Combe, and D. Gerlier.** 1993. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J. Virol. **67:**6025–6032.
17. **Offermann, M. K., J. Zimring, K. H. Mellits, M. K. Hagan, R. Shaw, R. Medford, M. B. Mathews, S. Goodbourn, and R. Jagus.** 1995. Activation of the double-stranded-RNA-activated protein kinase and induction of vascular cell adhesion molecule-1 by poly (I):poly(C) in endothelial cells. Eur. J. Biochem. **232:**28–36.
18. **Rota, J. S., K. B. Hummel, P. A. Rota, and W. J. Bellini.** 1992. Genetic variability of the glycoprotein genes of current wild-type measles isolates. Virology **188:**135–142.
19. **Rota, J. S., Z.-D. Wang, P. A. Rota, and W. J. Bellini.** 1994. Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains. Virus Res. **31:**317–330.
20. **Rota, J. S., J. L. Heath, P. A. Rota, G. E. King, M. L. Celma, J. Carabana, R. Fernandez-Munoz, D. Brown, L. Jin, and W. J. Bellini.** 1996. Molecular epidemiology of measles virus: identification of pathways of transmission and implications for measles elimination. J. Infect. Dis. **172:**32–37.
21. **Schneider-Schaulies, J., L. M. Dunster, F. Kobune, B. Rima, and V. ter Meulen.** 1995. Differential downregulation of CD46 by measles virus strains. J. Virol. **69:**7257–7259.
22. **Soilu-Hanninen, M., A. Hanninen, J. Ilonen, A. Salmi, and R. Salonen.** 1996. Measles virus hemagglutinin mediates monocyte aggregation and increased adherence to measles-infected endothelial cells. Med. Microbiol. Immunol. **185:**73–80.
23. **Stoltenborg, J. K., P. W. Tsao, H. J. George, P. J. Bouchard, E. J. Wexler, and E. A. Hausner.** 1994. A fluorescent cellular adhesion assay using insect cell produced human VCAM-1. J. Immunol. Methods **175:**59–68.
24. **Takaku, K., T. Sasada, T. Konobe, K. Onishi, S. Ueda, M. Takahashi, Y. Minekawa, T. Ogina, N. Suzuki, K. Yamanishi, K. Baba, and Y. Okuno.** 1970. Studies on further attenuated live measles vaccine. III. Selection of less reactive variants of CAM measles vaccine virus. Biken J. **13:**163–168.
25. **Vaporciyan, A. A., M. L. Jones, and P. A. Ward.** 1993. Rapid analysis of leukocyte-endothelial adhesion. J. Immunol. Methods **159:**93–100.
26. **Wyde, P. R., N. R. Attibele, and W. L. Kemp.** 1994. Infection of leucocytes by measles vaccine viruses Edmonston-Zagreb and Enders-Moraten has different consequences: potential mechanisms for increased vaccine efficacy or aberrant activity in field trials. Vaccine **12:**715–722.